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Research Article

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In vitro Evaluation of Total Phenol and Antioxidant Activity of *Achillea Millefolium* and *Stevia Rebaudiana*

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ABSTRACT

Aim of the present study is to compare antioxidant and total phenolic content of aqueous and ethanolic extract of Achillea millefolium, Stevia rebaudiana. Initially 1 gram of each plant sample was taken for preparation of ethanolic and aqueous extract. In estimation of total phenolic content of extracts from these plant, ethanolic extract shows 89.99 µg/ml of total phenol for Achillea millefolium and for Stevia rebaudiana ethanolic extract with 59.57 µg/ml. Similarly, to ensure antioxidant potential of extracts, total antioxidant capacity assay and DPPH scavenging potential was evaluated for these two extracts. In these studies, compared to aqueous extract, ethanolic extracts of Achillea millefolium and Stevia rebaudiana shows significant total antioxidant and DPPH scavenging potential. In DPPH scavenging activity, aqueous extract of stevia shows highest inhibition activity ranging from 86-89% inhibition and for Achillea millefolium 85%-88% inhibition at 10-50 10 to 50 µg/ml concentrations. Overall, ethanolic extracts shows significant activity from these plants. Based on experimental results, it was concluded that, need to further explore and purification of total phenol from these extracts of plants, may give platform for further explore of plant-based drugs and their avenues.

Keywords: *Achillea millefolium*; *Stevia rebaudiana*; Total phenol; Antioxidant

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INTRODUCTION

Achillea millefolium, commonly known as yarrow or common yarrow, is a flowering plant in the family Asteraceae. It is native to temperate regions of the Northern Hemisphere in Asia and Europe and North America. It has been introduced as a feed for livestock in New Zealand and Australia, where it is a common weed of both wet and dry areas, such as roadsides, meadows, fields, and coastal places. In Iran it can be found in the north, around the Alborz Mountain and in Azerbaijan, Lorestan, Isfahan, and markazi provinces [1]. In New Mexico and southern Colorado, it is called plumajillo (Spanish for 'little feather') from its leaf shape and texture.

In antiquity, yarrow was known as herbal militaris, for its use in staunching the flow of blood from wounds. Other common names for this species include gordaldo, nosebleed plant, old man's pepper, devil's nettle, sanguinary, milfoil, soldier's woundwort, thousand-leaf, and thousand-seal. Few studies reported for wound healing properties. For example, rabbit animal model used for wound healing by using hydroalcoholic extract of *Achillea millefolium*. Yarrow has been used as a traditional medicine by many cultures for hundreds of years. Yarrow tea is used as a diaphoretic remedy to treat fevers and colds in North America and the United States. In Germany, yarrow flowers are licenced as standard medicinal tea for the treatment of biliary and gastrointestinal disorders.

Stevia rebaudiana belonging to *Asteraceae* also known as "sweet herb", a native plant of Paraguay is used as a natural sweetener. *Stevia* is a perennial herbaceous plant that can grow upto 60-80 cm tall [2]. The leaves of this plant contain glycosides (steviosides and rebaudiosides) which are 100-300 times sweeter than sucrose. The glycoside, rebaudioside A present in *stevia* is 400 times sweeter than sucrose [3]. One of the major areas of uses of *stevia* is food supplement for diabetic peoples. There is no permanent drug for the treatment of diabetes and the recent oral hypoglycemic drugs have undesirable side effects on patients. It is proven that *stevia* controls blood sugar level and it is legally approved as food additives in Brazil, Korea and Japan [4]. But the major limiting factor for the large-scale production of *stevia* is the low percentage of germination capacity of *stevia* seeds [5].

Apart from, *Stevia* possesses some additional properties such as anti-hypertensive, anti-hyperglycemic and anti-human rotavirus. Consuming *stevia* on a regular basis will not produce any dental caries effect, this was confirmed in a research study [6]. A glucose tolerance test was performed before and after administration of the *stevia* leaf extracts. The results showed that treatment with *Stevia* resulted in an increase in glucose tolerance and a decrease in plasma glucose concentrations [7]. And it was shown recently that both steviol and stevioside produce a direct effect on beta cells in the islets of Langerhans of pancreas to release insulin. The authors concluded that this plant may have a potential use in the management of type 2 diabetes [8]. In South America and Japan have a history for the use of *stevia* sweeteners and there are no adverse effects recorded [9]. And the antioxidant activity of *stevia* is due to the presence of phenol and flavonoids. Phenols are important in the plant for normal growth and defense against infection and injury. The presence of phenolic compounds in injured plants have an important effect on the oxidative stability and microbial safety. Although phenolic compounds do not have any known nutritional function, they may be important to human health because of their antioxidant potency [6]. Based on available literature report, we opted these two plants from same family (Asteraceae), and compared to estimate total phenolic content, total antioxidant, and DPPH scavenging potential of *Achillea millefolium*, *Stevia rebaudiana* ethanolic and aqueous extracts.

EXPERIMENTAL SECTION

Plant Sample Collection

Stevia rebaudiana were collected from nursery at Saravanampatty, Coimbatore and *Achillea millefolium* collected from Nursery Garden at Ooty. The plant samples were maintained at room temperature for 2 days.

Preparation of Extracts

For preparation of extracts, both plant leaves were taken. Each 1 g of leaf was crushed by mortar and pestle using each 10 ml of 70% ethanol, and water and all four extracts were properly labelled and stored for further study.

Determination of Total Phenol

The total phenolic assay was determined using Folin Ciocalteu method with the following procedures. 20 ml of all four samples i.e., ethanol, and water extracts of two plant samples, each are taken in different tubes and all the tubes are made up to 1 ml with distilled water. To each tube 0.5 ml of Folin's reagent and 2.5 ml of 20% sodium carbonate was added. The tubes were then kept in dark for 40 mins. Then the absorbance value was estimated at 725 nm. The concentration can be determined now using the standard graph of gallic acid.

Evaluation of Antioxidant Potential of Extract

Total antioxidant capacity assay: Total antioxidant capacity assay is based on the principle of reduction of molybdenum (VI) to molybdenum (V) by the extracts and subsequent formations of green phosphate/molybdenum complex at acid pH 0.1 ml of the extracts at different concentrations [10].

DPPH radical scavenging activity assay: This method was measured by a modified spectrometer method. The principle of this method is that DPPH radical is scavenged by antioxidants through the donation of protons forming reduced DPPH. The solution loses colour depending on the number of electrons taken up. The colour changes from purple to yellow colour after reduction and the antioxidant activity is determined by the decrease of absorbance at 517 nm [11,12].

Statistical analysis: All the experiments were triplicated, and standard deviations were represented in that all data and experimental results.

RESULTS AND DISCUSSION

Solvent Extraction from Selected Plants

Due to Covid pandemic, we focussed only on the leaves of *Stevia rebaudiana* and *Achillea millefolium* for preparation of extract for further study. Figure 1 shows extraction of phytochemicals by using two different solvents such as ethanol and water for both selected plants.

Figure 1: Preparation of ethanolic and aqueous extracts from *Achillea millefolium* and *Stevia rebaudiana*

Determination of Total Phenolic Content

Figures 2 and 3 shows total phenolic content for Yarrow (*Achillea millefolium*) and *Stevia* (*Stevia rebaudiana*) using water, and ethanol as a solvent, phenolic content was measured by using gallic acid as the standard graph (Figure 3). By comparing total phenolic content, ethanolic extract shows highest concentration of total phenol (89.99 µg/ml) for *Achillea millefolium* and for *Stevia rebaudiana* ethanolic extract with 59.57 89.99 µg/ml. Overall, ethanolic extract shows significant concentration of total phenol from the selected plants compared to aqueous extract [13].

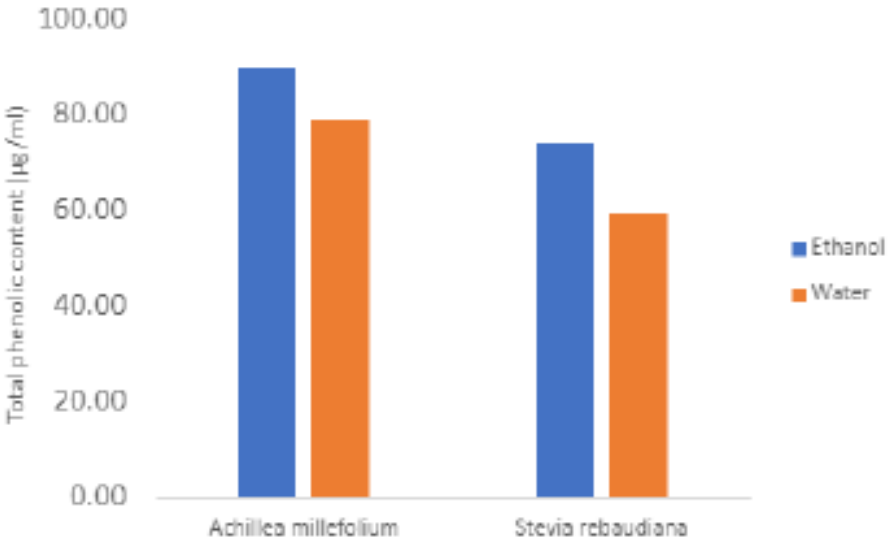

Figure 2: Total phenolic content of solvent extracts from *Achillea millefolium* and *Stevia rebaudiana*

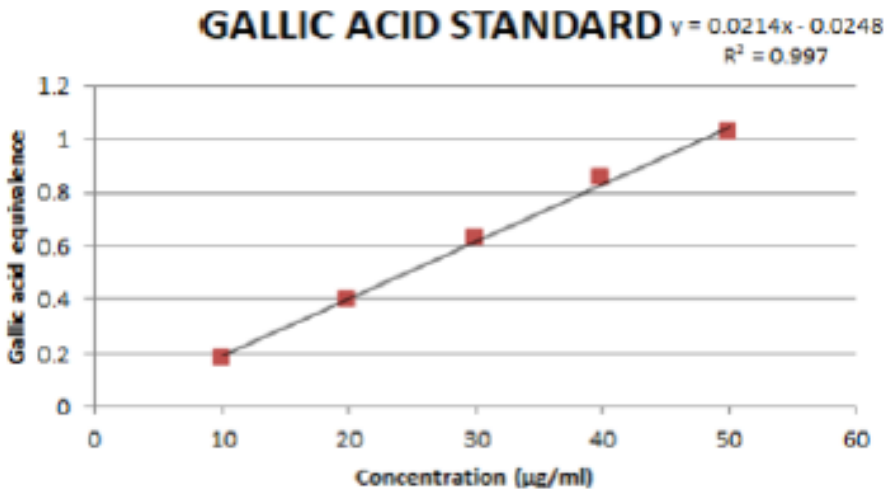

Figure 3: Standard graph for estimation of total phenolic content of extracts

Antioxidant Potential of Extracts

Total antioxidant capacity assay: Antioxidants are substances that can prevent or slow damage to the cells caused by free radicals. Total antioxidant capacity is the general antioxidant assay used to determine the presence of antioxidants present in the sample extracts. Antioxidant activity was reported for few chemical compounds in the article [13]. Here Figures 4 and 5 shows standard graph for estimation of total antioxidant capacity of both aqueous and ethanolic extracts of *Achillea millefolium* and *Stevia rebaudiana* were compared. Standard values of ascorbic acid used to estimate total phenolic content and has been found that ethanolic extract shows higher concentrations than the aqueous extracts of these two plants based on the equivalence of ascorbic acid.

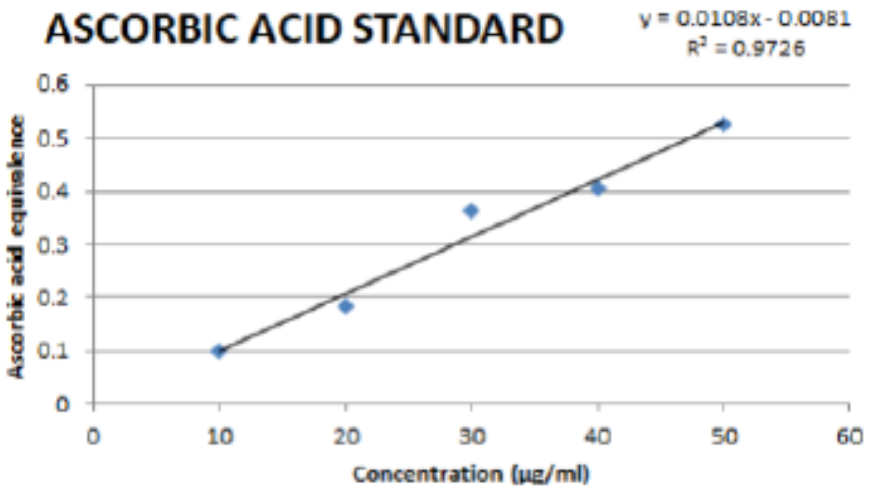

Figure 4: Standard graph for Ascorbic acid to estimate total antioxidant potential of extracts

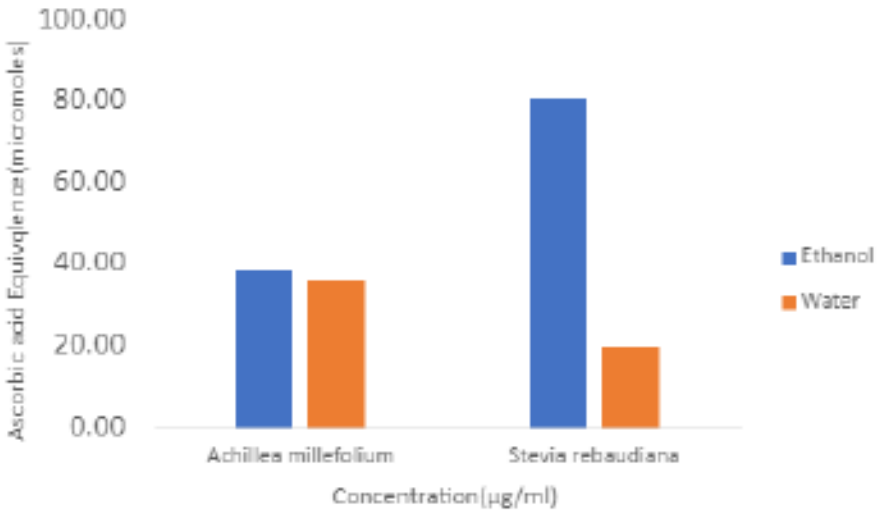

Figure 5: Total antioxidant capacity of Aqueous and ethanolic extracts of *Achillea millefolium* and *Stevia rebaudiana*

DPPH radical scavenging activity: Since DPPH assay is the most sensitive assay for determining the antioxidant property, the sample extracts of two plants *Achillea millefolium* and *Stevia rebaudiana* with different concentrations were taken and the assay performed. The standard DPPH assay always uses methanol or ethanol as a solvent. In this assay, Trolox equivalency is used as standard values for determining the presence of antioxidants. Trolox equivalency was most often measured using the ABTS decolorization assay. The absorbance values were recorded at 517 nm with varying concentration of the sample extracts. Figure 6 shows Trolox standard graph for estimation of total antioxidants capacity. The scavenging activity was checked for various plant extracts when compared with butyr hydroxyanisole (BHA) as a standard which was reported in the article [14]. Similarly, effect of growth regulators on *Stevia rebaudiana*, phenols, flavonoids and other phytochemical accumulation, and antioxidant activity studied [15,16].

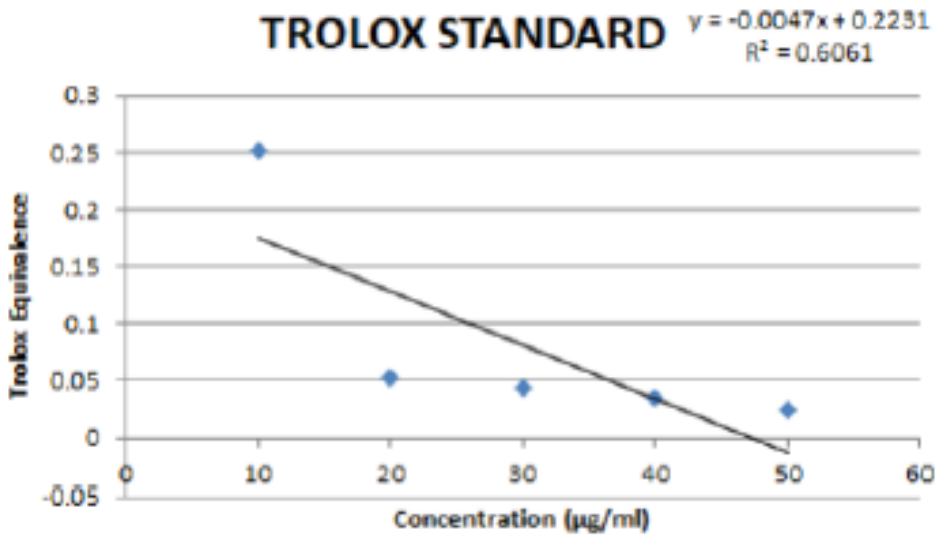

Figure 6: Trolox standard for estimation of reducing potential of extracts by DPPH assay

From the Figure 7 shows, ethanolic extract *Achillea millefolium* shows higher percentage of inhibition compared to *Stevia rebaudiana* ethanolic extracts even starting from 10-50 µg/ml concentrations. Percentage inhibition starts from 84% to 91% for *Achillea millefolium* and for *Stevia rebaudiana* it ranges from 66% to 88% of inhibition.

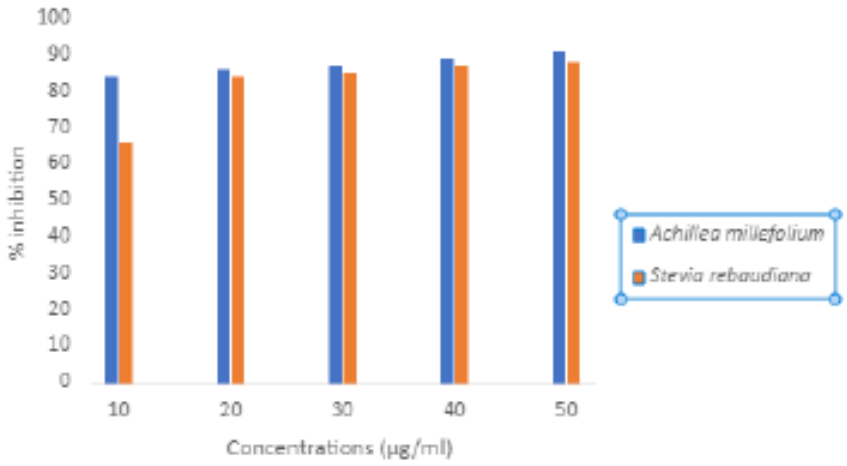

Figure 7: % Inhibition of ethanolic extracts of *Achillea millefolium* and *Stevia rebaudiana*

Similarly, Figure 8 shows % inhibition of DPPH by aqueous extract of *Achillea millefolium* and *Stevia rebaudiana*. In this figure, *Stevia rebaudiana* aqueous extract shows significant % inhibition of *Achillea millefolium* at 10 to 50 µg/ml concentrations. It was noticed that, 85%-88% inhibition for *Achillea millefolium*, 86%-89% inhibition for *Stevia rebaudiana*.

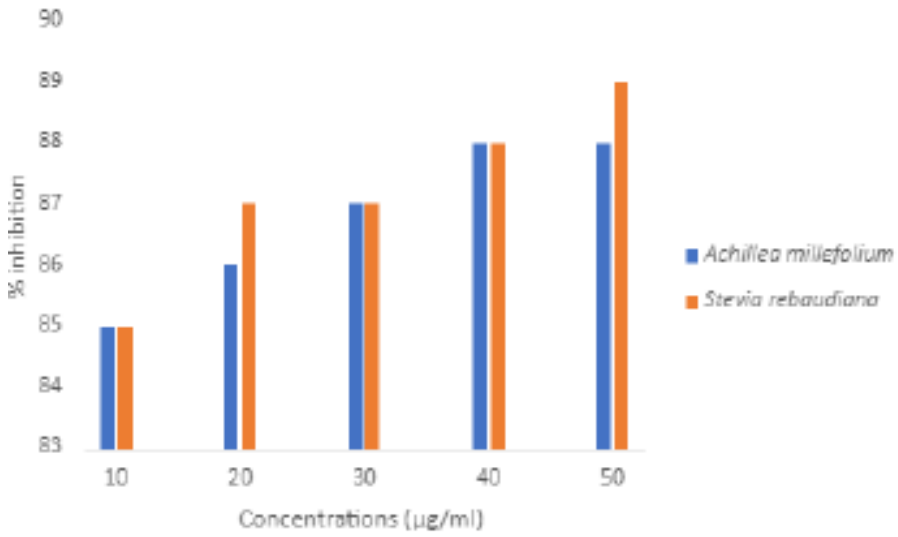

Figure 8: % Inhibition of aqueous extracts of *Achillea millefolium* and *Stevia rebaudiana*

CONCLUSION

Initially, total phenolic content of aqueous and ethanolic extracts of yarrow (*Achillea millefolium*) and *Stevia* (*Stevia rebaudiana*) was measured by using gallic acid as the standard graph. Based on total phenolic content of leaf extract, ethanolic extract showed significant results in terms of total phenol content and total antioxidant potential, DPPH scavenging activity. For example, In DPPH scavenging activity, aqueous extract of *stevia* shows highest inhibition activity ranging from 86%-89% inhibition and for *Achillea millefolium* 85%-88% inhibition than the ethanolic extracts at 10-50 10 to 50 µg/ml concentrations. So, it gives scope for further exploration and purification and application of total phenol from extracts of these *Achillea millefolium* and *Stevia rebaudiana*.

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